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EFFECTS OF DDT ON STEROID METABOLISM AND ENERGETICS IN BOBWHITE QUAIL (COLINUS VIRGINIANUS)

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Abstract:

Female bobwhite quail (Colinus virginianus) fed low levels (5 ppm) of DDT in their diets showed increased enzyme induction in the liver and consequently a significant increase in steroid (progesterone) metabolism (a mean of 54% conversion of progesterone to its more polar metabolite in experimentals compared to a mean of 24.1% conversion in controls). The mean conversion of testosterone to its polar metabolite (25.1%) in experimental males was greater than in the controls (mean 18.1%) but was only significantly greater in certain males. There was a correlation ($r = 0.7$ $P<0.01$) between percent body weight of testes and percent conversion of testosterone to its metabolites (the smaller the testes the greater the conversion). A correlation ($r = 0.66$ $P<0.02$) was also found between circulating levels of DDE, DDT, and testes size (the higher the pesticide level the smaller the testes).

Dietary levels of DDT (10,50,100,150 ppm) affected the energetics (oxygen consumption) of bobwhite quail. All DDT-treated birds had a higher metabolic rate than the controls at all ambient temperatures tested except 30 C. After acclimation to an ambient temperature of 5 C for 10-13 weeks, birds on 100-ppm DDT diets had a significantly ($P<0.01$) higher metabolic rate than controls. After one week of exposure to -18 C there was a significant ($P<0.02$) increase in thyroid weight in the birds on 100-ppm diets. Birds on 100-ppm diet exposed to extreme cold for 1 week died of DDT toxicity.

Data on tissue residue levels, weight changes, I^{131} uptake by the thyroid, and adrenal changes are also presented. The ecological significance of the synergistic effect of DDT and cold stress on the bobwhite quail is discussed.

Numerous studies have been carried out on the toxicity of various pesticides based on gross biological phenomena observed in living organisms. Only in recent years has attention been focused on the effect of pesticides on metabolic functions that can be examined at the enzyme level. The administration of organochlorine insecticides to animals of various species stimulates both the hepatic microsomal oxidation of drugs and the microsomal hydroxylation of steroids including progesterone and testosterone (7,14,24).

Widespread and severe decreases in populations of several avian species have been noted throughout the world in the past 15 years (5,11,26,33). Correlations between these decreases in populations and the increased use of (DDT) pesticides were observed, but the concentration of pesticide residues found in these species was low compared with a toxic dose (28). There is also evidence that gross blockage of the

reproductive systems does not occur except at concentrations approaching toxic doses (1,18). Decreased populations might result from more subtle effects on the breeding cycle (in social dominance) caused by changes in hormone levels and behavior. Peakall (24) stated "that if normal regulation of populations depends on a subtle balance of hormone concentrations and related feedback mechanisms associated with population density, it would not be surprising to find that agents affecting this balance cause declining populations." Conney et al.(8) suggested that the stimulating effect of halogenated hydrocarbon insecticides on steroid hydroxylation possibly explained the effect of these pesticides as exemplified by decreased fertility in experimental animals. DDT not only affects reproduction by altering steroid metabolism but Welch et al.(32) suggested that DDT could act directly as an estrogen. Ecobichon and Saschenbrecker (10) demonstrated that DDT-treated white leghorn cockerels 5 weeks old showed a marked reduction in both comb and testicular development when compared to control birds after 32 weeks of treatment. Once again these results could be taken to indicate that DDT has an estrogenic effect (masking the testosterone in juvenile birds with low testosterone levels).

Since DDT and its metabolites are stored in the fats, any factor increasing the energy requirement of the bird (cold stress) could result in higher circulating levels of DDT and its metabolites. Therefore, not only is it important to know the effects of DDT on steroid metabolism but it is equally important to know the effects of DDT and cold stress on the energetics of the bobwhite quail.

This study was concerned with: (A) the effect of low doses of DDT (5 ppm technical grade--Diamond Shamrock Chemical Co., Texas) on steroid function and metabolism (progesterone and testosterone) in bobwhite quail, (B) the effect of DDT on the reproductive capabilities of bobwhite quail, and (C) a comparison of the effects of cold stress on DDT-fed and control quail by studying their metabolism (oxygen consumption) over a wide range of ambient temperatures.

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Methods and Materials

DDT and Steroid Metabolism

Twenty-eight yearling pen-raised quail with a mean weight of 167.2 g were divided into groups (3 females and 7 males fed the control diet, 4 females and 14 males fed the experimental diet containing 5 ppm DDT). All diets consisted of Purina Game Bird Startena either mixed with corn oil only (Mazola Brand, control diet) or with DDT dissolved in corn oil. The birds were maintained on the diet from 30 to 70 days starting 30 June 1969. Except for 2 birds that were sacrificed in December (when the testes were regressed) all birds were sacrificed during the 70-day period. The procedure used for enzyme extraction was essentially that of Conney and Klutch (7), while that for steroid analysis was that of Peakall (24).

The testes from each of the male birds were weighed to the nearest 0.01 g. Residue levels were determined in the liver by gas chromatography (20).

DDT and Energetics

Methods for determining oxygen consumption and thermal conductance are described by Voss (31). The birds used in the different metabolic experiments consisted of 2 groups which were then further divided into subgroups.

Group 1 consisted of 1-month-old male and female bobwhites hatched on 2 June 1970. The birds were kept indoors for 10 days, then transferred outside into 5 wire cages of 6.1 m x 6.1 m x 11.2 m each. In mid-September the quail were placed in 5 cages of 9.2 m x 10.7 m x 14.3 m, 7 quail to a cage. Each of these cages contained a box with an opening so that the quail could escape inclement weather. Except for the first 10 days after purchase, the birds were outside and, therefore, acclimated to ambient conditions of temperature and photoperiod.

On 1 August, each of the 5 subgroups was placed on a diet containing different amounts of technical grade DDT purchased from Diamond Shamrock Chemical Corp. (0,10,50,100, and 150 ppm DDT). Technical grade DDT was dissolved in 100% pure Mazola brand corn oil.

All birds were sacrificed following the metabolic tests, there being no fatalities in any of the subgroups over the 211- to 242-day period. The livers and brains were weighed and enough fat tissue removed to be used for residue analysis by gas chromatography. These tissues from 4 birds from each subgroup were analyzed for DDT and its metabolites by the method proposed by Nauman (20).

Group 2 consisted of 21 4-month-old quail of both sexes. On the first of September 1971 these birds were divided into 3 subgroups of 7 birds each. The birds were then placed in individual cages (18 x 41 x 23 cm) inside a Sherer Dual Jet Model 810 environmental room capable of maintaining an ambient temperature $\pm$ 1.0 C of the desired temperature over an ambient temperature range of from -20 to 60 C. The birds were allowed to adjust to the chamber for 1 month. During this adjustment period the birds were maintained at an ambient temperature of 20 C and on a 12-hr photoperiod between 0830 and 2030 hr. On 2 October 1971, the ambient temperature in the environmental room was dropped to 5 C, and 2 of the 3 subgroups were placed on DDT diets of 10 ppm and 100 ppm respectively; the other subgroup was maintained as a control. The diets were mixed as previously described. The birds were maintained under these experimental conditions until 31 January 1972. The oxygen consumption at 5 C for all 3 subgroups was determined between the 2nd and 3rd week and again between the 10th and 13th week to determine what effect DDT and long-term acclimation to low ambient temperature might have had on the energetics of bobwhite quail. All birds were weighed periodically during the test period to determine if there were significant differences in ability to maintain weight between the 3 subgroups.

The potential effect of extreme cold stress was tested. On 31 January 1972, the temperature in the environmental room was dropped to -18 C, and 5 control birds, 5 birds on the 10-ppm-DDT diet, and 4 birds on the 100-ppm diet were injected intraperitoneally with 1μc of iodine (I^{131}) in physiological saline. The birds were maintained at this ambient temperature for 1 week; surviving birds were then sacrificed and thyroid weights and activity (I^{131} incorporation) determined. Brain levels of DDT and its metabolites were determined for 4 birds from each of the subgroups (control, 10, and 100 ppm DDT) by the method mentioned previously. The thyroid samples were prepared for analysis in the liquid scintillation spectrometer as described by Nauman (20). All iodine uptake values were adjusted so that they were based on a 28-hr exposure (time when first bird died). This enabled us to compare all birds.

Two LD_{50} tests were conducted on yearling birds by exposing them to diets ranging from 500 to 1500 ppm. In the first test, concentrations of 500, 600, 700, and 800 ppm DDT in the diet were used.

Results

DDT and Steroid Metabolism

Liver microsomal enzyme extracts from DDT-treated females all induced significantly ($P<0.001$) greater conversion of progesterone to its more polar metabolites than did extracts from control females (54.0% conversion in experimental females compared to 24.1% in the control females, Table 1). The mean conversion of testosterone in control males (18.8%) to its more polar metabolite was less than in the experimental males (25.4%) but there was no significant difference. Liver enzyme extracts from certain DDT-treated individuals showed a greater conversion of testosterone to its more polar metabolites than the control (Table 1). In males there was a correlation ($r = 0.7$, $P<0.01$) between testes weight and percent conversion of testosterone to its metabolites (both polar and less polar). Increased conversion of testosterone to its metabolites was inversely related to testes weight (Fig. 1).

The major pesticide residues in the liver, and thus circulating in the blood, were p,p' DDT (♂ 0.09 ppm, ♀ 0.10 ppm) and p,p' DDE (♂ 0.59, ♀ 0.26) though there were traces of o,p' DDE, o,p' DDT and p,p' DDD.

When 2 male birds with extreme values are omitted from the least squares analysis, a correlation ($r = 0.66$, $P<0.02$) between p,p' DDT in the liver and percent body weight of the testes (Fig. 2) was obtained. The greater the residue levels the smaller the testes. A similar correlation was found between o,p' DDT in the liver and percent body weight of the testes.

DDT and Energetics

The oxygen test results over a range of ambient temperatures (5 - 40 C) (Group 1) in the quail fed DDT varied from the control birds

at all ambient temperatures except 30 C (Fig. 3). At the lower temperatures the differences in metabolic rate (O_2 consumption) of the five subgroups were significant only in that the experimentals were all higher than the controls (Fig. 3). As the thermoneutral zone (30 - 40 C) was approached the lines converged, with little difference among the dietary groups. The differences were greatest at the low ambient temperatures.

Above the upper critical temperature there is a considerable divergence of the means. One control bird tested at 45 C became hyperactive after only 15 min in the chamber, and over the next 5 min changed from using 126 cc/hr to 330 cc/hr of oxygen. Forty degrees centigrade seemed too severe for the experimental birds because 5 collapsed in the chamber and had to be removed before any readings were taken. Repeated tests were made on these birds and oxygen consumptions were obtained at 40 C; however, the time these bobwhites spent in the chamber ranged from only 30 to 75 min. None of the controls collapsed at 40 C. At the lower test temperatures all quail could spend an indefinite time in the test chamber, that is, until starvation or dehydration. All groups exhibited gular flutter and loss of moisture (collecting in the outlet tube from the chamber) at 40 C, indicating the humidity in the chamber was high since dry air was entering the chamber. Despite gular flutter and moisture loss, oxygen consumption of the control birds (mean of 1.17 ± 0.099 cc O_2/g/hr) at 40 C was of the same order as that of the control birds at 30 C (1.12 ± 0.103 cc O_2/g/hr) and 35 C (1.08 ± 0.048 cc O_2/g/hr) indicating that they were still within their zone of thermoneutrality at 40 C. The experimental birds on 10-, 50-, 100-, and 150-ppm-DDT diets used 1.39 ± 0.227, 1.40 ± 0.214, 1.30 ± 0.237, and 1.40 ± 0.524 cc O_2/g/hr respectively at 40 C. These values are not only higher than the controls at 40 C but also higher than the experimentals at 30 and 35 C, indicating that the upper critical temperature had been decreased. A greater variation in the oxygen consumption of the experimentals at 40 C than in the controls also suggested a lowering of the upper critical temperature in the DDT-treated birds; in thermal neutrality variation is at a minimum (19). The thermoneutral zone of the control birds was over a 10 C range, approximately between 30 and 40 C, while in the DDT-fed quail the thermoneutral zone was within a 5 C range, approximately from 30 to 35 C for all the treated groups. The body temperature of all quail remained relatively constant between ambient temperatures of 10 and 35 C. At 40 C the body temperature of all birds increased though that of the controls increased the greatest amount, whereas, at an ambient temperature of 5 C only the body temperature of the controls dropped significantly.

Thermal conductance (cc O_2/g/hr/C) was not significantly different among the 5 subgroups at any ambient temperatures except 40 and 35 C. At 40 C the thermal conductances of birds on diets of 10 (0.44 cc O_2), 100 (0.46 cc O_2), and 150 (0.52 cc O_2) ppm DDT are significantly different ($P<0.05$) from controls (0.34 cc O_2). Though the subgroup on 50 ppm DDT had a mean thermal conductance higher than the controls, it is not significant ($P>0.05$). At an ambient temperature of 35 C the only experimental group significantly different ($P<0.05$) from the controls (0.16 cc O_2) with regard to thermal conductance was the subgroup on

the 150-ppm-DDT diet (0.20 cc O_2). There were no apparent trends among the experimental birds except at 40 C where increased DDT in the diet was correlated with increased thermal conductance.

In an attempt to determine if there were synergistic effects between long-term exposure to a moderately low ambient temperature (5 C) and DDT, we compared the oxygen consumption (at 5 C) of Group 1 quail on the control, 10-ppm, and 100-ppm-DDT diets to the oxygen consumption of Group 2 quail (on similar diets) which were acclimated to ambient temperatures of 5 C and tested at 5 C. Several differences and similarities between energetics of Group 1 and Group 2 quail at 5 C are apparent in Figure 4:

1. The birds on 10- and 100-ppm diets, whether acclimated or not, had a higher metabolic rate than the controls.

2. There was no significant difference in oxygen consumption between the acclimated (Group 2) and nonacclimated (Group 1) controls.

3. There was no significant difference in oxygen consumption between Group 1 and Group 2 quail on 10-ppm diets.

4. Group 2 quail on a 100-ppm-DDT diet had a significantly higher ($P<0.01$) oxygen consumption at 5 C than did Group 1 quail on the same diet and at the same temperature.

5. Group 2 birds on the 100-ppm-DDT diet had a significantly higher ($P<0.01$) oxygen consumption than did Group 2 and Group 1 controls at 5 C.

6. There was no significant difference in oxygen consumption between Group 2 birds (acclimated 2-3 weeks) and Group 1 birds on any of the diets.

Because the Group 2 quail on the 100-ppm DDT diet had a higher oxygen consumption than either Group 1 or Group 2 quail on any of the other diets, and increased oxygen consumption (metabolic rate) can sometimes be correlated to increased thyroid activity, thyroid activity (I^{131}) incorporation was determined at extremely low ambient temperatures (-18 C) (Table 2). All test birds, though given ad libitum food and water, lost weight over the 1-week test period. Controls dropped from a mean weight 197.3 $\pm$ 7.7 g at the start of the low-temperature test to a mean weight of 159.9 $\pm$ 13.3 g at the end of the test period, while those on 10- and 100-ppm-DDT diets dropped from 203.1 $\pm$ 5.5 g to 170.8 $\pm$ 8.2 g and 203.3 $\pm$ 3.6 g to 170.3 $\pm$ 2.6 g respectively. The weight change in the 100-ppm-DDT subgroup was at a mean rate of 12.1 g per day while in the controls and 10-ppm birds it was 5.3 and 4.6 g per day respectively.

In the 100-ppm-DDT subgroup, there was a significant ($P<0.02$) increase in thyroid weight expressed either directly in grams or indirectly as percent body weight as compared to the controls and

10-ppm-DDT subgroups (Table 2). No significant difference in thyroid weight was evident between the controls and 10-ppm-DDT subgroups. Iodine (I^{131}) activity expressed either as counts per minute or as percent of initial injection in the thyroid after 28 hr increased significantly ($P<0.01$) in the birds on 100-ppm-DDT diets over those on control and 10-ppm-DDT diets. There was no significant ($P>0.05$) difference between any of the groups when percent of initial injection of iodine incorporated into the thyroid per gram of thyroid was considered, though incorporation per gram was greatest in the birds on 10-ppm DDT, medium in the 100-ppm-DDT subgroup, and lowest in the control subgroup. During the 1-week low-temperature test period all birds sat in their cages with their feathers fluffed in order to minimize heat loss. Considerable shivering was noticed in all birds; shivering being especially prominent in the birds on 100-ppm-DDT diets 1 day prior to death. This may also have been related to DDT intoxication.

Mean hematocrits for the 5 subgroups (control; 10-, 50-, 100-, and 150-ppm-DDT diets) in Group 1 were found to be 38.6, 42.1, 42.1, 42.6, 41.5% respectively. This 3 to 4% increase of the experimentals over the controls was significant ($P<0.05$).

Trends in adrenal histology were apparent:

1. Control female adrenals were heavier than control male adrenals; whereas experimental females had lighter adrenals than male experimentals on every diet but 150-ppm DDT.

2. Control males had a higher percent cortical tissue (60.4%) and lighter adrenals than experimental males (53.1%).

3. Control female adrenals are heavier and have a greater percent cortex (67.9%) than experimental females (55.8%).

In Group 1, analysis of fat, brain, and liver tissue by gas chromatography showed that the metabolite most prominent in these tissues was DDE (Table 3). DDT was not detected in the 50-ppm sub-groups fat tissue in measurable amounts. The fat residues for the 50-ppm subgroup may not be representative because of the small amounts of fat which were removed from each bird. No traces of DDT were found in the control birds. DDD appeared only in liver tissue of birds fed 150 ppm DDT. Brain tissue levels of DDE were low in all subgroups in Group 1. Fat DDE levels increased in substantial amounts with the increasing concentrations of DDT in the diet.

A quail that died after 17 days on 800-ppm-DDT diet was dissected for tissue analysis. Fat residues were 18.23 ppm DDT, 12.7 ppm DDE, and 3.92 ppm DDD. The liver contained 77.6 ppm DDD and 103.0 ppm DDE. Brain levels registered 51.9 ppm DDT and 29.5 ppm DDE. Only brain levels showed a great increase compared to the figures for the 4 experimental subgroups in Group 1 on lower concentrations. In Group-2 quail the brain levels of p,p'DDE increased from a mean of 0.551 ppm in controls to a mean of 2.43 and 73.93 ppm in the 10- and 100-ppm subgroups respectively, while p,p' DDT increased from not detectable in

controls to a mean of 0.355 and 19.25 ppm in the 10- and 100-ppm-DDT diet subgroups respectively.

The liver weights expressed as percent total body weight were higher in all Group 1 experimental birds (10-, 50-, 100-, and 150-ppm-DDT diets, Table 4). There was no significant difference in percent body weight of the liver between the controls and birds on 10-ppm-DDT diets, whereas in the subgroups on 50-, 100-, and 150-ppm-DDT diets it was significantly ($P>0.05$) heavier than in either the controls or 10-ppm-DDT birds. The brain weight expressed as percent total body weight for all subgroups within Group 1 remained relatively constant for all birds.

The LD_{50} for bobwhite dying within a period of 4 days was 1150 ppm DDT in their diet.

Discussion

DDT and Steroid Metabolism

Significant information on effect of insecticides and other compounds on rate of metabolism of in vitro steroids, compared with biological activity of in vivo steroids, is only preliminary. Little is known about the effect of DDT on steroid metabolism and enzyme induction in birds and especially game birds; Peakall (23,24) having made the only published reports. The effect of environmental doses of DDT on bird behavior (especially reproductive behavior) is virtually unknown.

The results suggest that in bobwhite quail, testes size may be related to the rate of induction of liver enzymes by DDT. DDT-treated males with smaller testes (which may indicate subordinate males with lower testosterone levels) showed the greatest conversion of testosterone to its more polar metabolites. DDT may have an estrogenic effect (3,25,32), thus converting subordinate-male (low testosterone levels) to female-type responses and explaining somewhat the similarity in response (in steroid metabolism) between females and males with regressed testes. Juvenile male quail and male quail with regressed testes during refractory period may be converted to and maintained in a female-like state due to low testosterone levels and the possible estrogenic effect of DDT. Concomitant with this is the increased enzyme induction (increased conversion of progesterone and testosterone to their more polar metabolites) due to DDT in females and subordinate males suggesting lower circulating levels of these hormones.

Peakall (23) has demonstrated in ring doves that DDT caused a decrease in circulating estrogen levels, a thinning of egg shells, and a delay in egg laying. Though it is unlikely that bobwhite quail would encounter high environmental levels of DDT in their diet, our study suggested that low environmental doses of DDT could be stored in the fats during the summer months when the birds are feeding partially on insects. During the winter months when the birds metabolize fats (see section on quail energetics) circulating levels of DDT and its metabolites could be high enough to enhance enzyme induction and thus steroid metabolism. This would be especially true during the winter months since the testes

are in a regressed state. The smaller the testes the greater the conversion of testosterone to its metabolites. The decreased testosterone combined with the estrogenic effect of DDT could maintain the males in a female state, while the increased metabolism of estrogen and progesterone could account for delayed or complete lack of egg laying. This could explain why some adult birds with high tissue residues of DDT fail to breed.

DDT and Energetics

Quail are widely distributed in North America, from southwestern Wyoming south to eastern Mexico (2) and over most of the continent to the east, and are faced with many natural environmental stresses, especially a wide range in ambient temperature. The bobwhite (a relatively good homeotherm) maintains a relatively constant body temperature demonstrating a remarkable control and coordination over the mechanisms of heat production and heat loss. According to Scholander's et al. (27) adaptation of Newton's law of cooling ($MR = C [T_B - T_A]$) heat production can be equated to metabolic rate (oxygen consumption) and heat loss to thermal conductance (C) times the body temperature minus the ambient temperature. Therefore, by studying the energetics (oxygen consumption) of an animal it is possible to get some insight into how this animal adapts to ambient temperature stress and what effect DDT might have on its ability to survive under various ambient temperatures.

As indicated by the measurement of body temperatures over an ambient temperature range of 10 - 35 C the bobwhite can maintain a mean body temperature of 41.5 C. At ambient temperatures of 5 C the birds undergo a slight hypothermia (T_B 40.6) whereas at an ambient temperature of 40 C body temperature becomes hyperthermic (43.4 C). The zone of thermal neutrality ($\approx$30 - 40 C) for a bobwhite approximates that found by Brush (4) for the California quail, (Lophortyx californicus, 27 - 37.5 C). The mean oxygen consumption (1.12 cc O_2/g/hr) agrees closely with the value predicted by the Lasiewski-Dawson (16) equation for a 200-g nonpasserine bird (23 Kcal/day); the equation is $M = \log 78.3 + 0.723 \log W$; where M equals metabolic rate and W equals weight (15). The zone of thermal neutrality, therefore, extends over approximately 10 C (30 - 40 C) and is high when compared to most other birds. A high zone of thermal neutrality is usually assumed to be an adaptation to high ambient temperature and supports Darlington's (9) theory that bobwhite quail evolved in the warm climate of southern North America.

The most generally employed model for describing the metabolic response of homeotherms at rest to ambient temperatures is based on ideas related to Newton's law of cooling, with thermal conductance being minimal and uniform below the thermal neutral zone, with body temperature remaining uniform over a very wide range of ambient temperatures and with clearly defined upper and lower critical temperatures. By comparing the shape of the curve relating oxygen consumption to ambient temperature (Fig. 3) to the values for thermal conductance, it is evident that below an ambient temperature of 20 C the thermal conductance (mean of 0.064 cc O_2/g/hr/C) remains relatively constant and the regulation of body temperature is due to increased chemical thermogenesis. The mean thermal

conductance of quail below 20 C approximates that predicted by Lasiewski et al. (15) for a 200-g bird (0.06 cc O_2/g/hr/C). The increased thermogenesis in the bobwhite was presumably due entirely to shivering because non-shivering thermogenesis has not yet been demonstrated in birds (19). The increasing values for thermal conductance and oxygen consumption between ambient temperatures of 20 C and 30 C (the lower critical temperature) indicated that the maintenance of a constant body temperature over this temperature range is accomplished by both increased thermogenesis and insulative changes in the shell (peripheral heterothermy, feather arrangement, tucking beak under feathers, and sitting on unfeathered legs). Thus, the bobwhite does not follow Newton's law of cooling (constant thermal conductance below thermal neutrality). In the bobwhite quail there is a gradual transition from regulation of body temperature primarily through control of insulative changes (thermal conductance) to regulation primarily through thermogenesis. As the ambient temperature approached the body temperature there were increasing amounts of moisture in the air leaving the metabolic chamber (determined by the rate of color change in drierite). As Brush (1965) pointed out in the California quail, as the T_A increased above the lower critical temperature, evaporation was responsible for progressively greater amounts of heat dissipation, whereas at ambient temperatures below the lower critical temperature it accounted for only about 5% of the total heat loss. The mechanism for evaporative water loss in the bobwhite is through a combination of panting and gular flutter which Lasiewski et al. (15) have found to be an extremely efficient means of heat loss; birds can lose better than 100% of the heat produced or gained from the environment by this method. At an ambient temperature of 40 C the bobwhite quail underwent a slight hyperthermia (T_B 43.4 C). This ability to withstand a slight hyperthermia aids the bird in heat loss since the temperature gradient from the bird to the environment is greater and heat dissipation can occur by conduction and convection rather than by the energy-requiring panting and gular flutter. This hyperthermia accounts somewhat for the high upper critical temperature of the bobwhite.

Below the zone of thermal neutrality (30 - 40 C) the maintenance of a constant body temperature is an energy-requiring process and anything that would increase this energy requirement would be detrimental to the birds, especially if food were scarce.

In this study the oxygen consumption of all the experimental birds (10-,50-,100-,150-ppm-DDT diets) was higher than that of the controls at all test temperatures except 30 C. Ozburn and Morrison (22) working with white mice, and Jefferies and French (13) working with pigeons, also found that DDT in the diet increased the oxygen consumption of these animals.

By comparing the oxygen consumption of the controls to that of the birds on various DDT diets over an ambient temperature range of 5 C to 40 C it becomes obvious that the greatest differences exist at the extreme ambient temperatures of 5 and 40 C. In fact, it is at an ambient temperature of 40 C that the greatest differences in oxygen consumption occur. Not only was the oxygen consumption of experimentals higher at 40 C than that of the controls but it was also higher than

the experimentals at 35 C, suggesting that the upper end of the zone of thermal neutrality was shifted down at least 5 C (to 35 C). This was evident because the experimental birds collapsed in the test chamber at 40 C. The lowered upper critical temperature in the DDT-fed quail might well be a result of the greater heat production within the bird due to the increased metabolic rate combined with the inability to withstand as great hyperthermia as did the controls. Though metabolic rate was higher in the DDT-fed birds, body temperature was less, indicating that the experimental birds were losing heat faster. As mentioned previously, the major means of heat loss at high ambient temperatures is evaporative water loss (panting and gular flutter). Calder and Schmidt-Nielsen (6) have shown that increased oxygen consumption with panting and gular flutter in pigeons results in alkalosis of these birds under heat stress. Perhaps increased alkalosis was causing the experimental quail to collapse in the metabolic chamber.

At an ambient temperature of 5 C the control birds had lower body temperatures ($\bar{X}$ = 40.6 C) than the experimentals ($\bar{X}$ = 41.5 C). Below an ambient temperature of 20 C the thermal conductance of all the birds was minimized (maximum insulation). Because all the birds had the same insulating capacity, thermal conductance was the same. Therefore, a lower metabolic rate (lower heat production) in the control birds would result in a lower body temperature.

The increased hematocrit in all experimental birds can be correlated to increased oxygen requirement. It is obvious from the metabolic results that there is a threshold level of DDT in the diet that brings about the increased metabolic rate, and the metabolic rate does not increase with increased dietary levels of DDT above the threshold level in unacclimated birds.

Several hypotheses for increased oxygen consumption have been set forth:

1. Increased enzyme induction in the liver brought about by chlorinated hydrocarbons (24) could cause an increased oxygen consumption in the experimental quail. We found increased induction of liver enzyme on dietary levels of DDT as low as 5 ppm and significant increases in liver weight in dietary levels of 50 ppm or better. If the increased O_2 consumption was due to the enzyme, induction increase should be constant at all ambient temperatures. Our results show that the increase in O_2 consumption was not constant at all ambient temperatures thus rejecting the increased enzyme induction hypothesis.

2. Since DDT's introduction as a poison, it has been known to act on the nervous system (30). Thus, the oxygen increase could also result from increased nervous activity caused by sublethal amounts of DDT. Our birds were tested in a dark chamber (birds sit still) so increased nervous activity was unlikely. Liver levels can be used to indicate the amount of pesticide circulating through the body and hence available to enter the central nervous system (10). The residue levels in the livers of the treated birds were low except for the 150-ppm birds.

3. The third hypothesis is that DDT causes an increased thyroid activity which in turn brings about an increased metabolic rate. It is also known that exposure to low temperature causes increased thyroid activity in birds (29).

To test this we acclimated Group 2 birds (control; 10- and 100-ppm diets) for at least 10 weeks to a moderately low ambient temperature (5 C) and once again measured their metabolic rate at 5 C. We now found that the birds on 100-ppm DDT had significantly higher O_2 consumption than the controls, whereas O_2 consumption in the acclimated controls was no different than in the unacclimated controls. This supports the hypothesis that it is DDT and not just cold stress that enhanced thyroid activity. The birds on either of the diets did not lose significant amounts of weight over the 4-month test period, suggesting that survivability was the same.

To test this hypothesis further we injected I^{131} into the Group 2 birds and lowered the T_A to -18 C for 1 week, at the end of which the birds were sacrificed and thyroid activity determined. The significantly greater weight of the thyroid of 100-ppm DDT birds combined with the fact that I^{131} activity per gram was only slightly higher than the controls suggests greater secretion of thyroxine. Our results agree with that of Jefferies and French (13) who found a significantly increased O_2 consumption correlated with increased thyroid weight in pigeons after 11 weeks on the various DDT diets. Their lowest dietary level of DDT was as high as our highest. Of the 3 hypotheses set forth the latter is the most acceptable, though it is possible that the increased oxygen consumption is due to a combination of all 3 (increased enzyme induction, nervousness, and thyroid activity).

In the present study, the only birds dying of DDT poisoning were those used in the LD_{50} test and the Group 2 birds on 100 ppm DDT diets exposed to -18 C. We found an LD_{50} of 1150 ppm for 1-year-old bobwhite quail fed over a 4-day period. This values agrees closely with that found by Hill et al. (12) (1170 ppm DDT) for subadult (weight 158.0 g) quail.

Considering the high p,p' DDT and p,p' DDE residues in fat tissue of birds on a 100-ppm-DDT diet for several months (238.1 ppm and 924.5 ppm respectively), the large weight loss (12 g/day) of 100-ppm-DDT birds at ambient temperature of -18 C and the high metabolic rate of these birds, it becomes apparent why these birds died of DDT poisoning. The mobilization of these fat stores in response to low temperature and elevated metabolism due to DDT, increased the circulating amount of p,p' DDT and p,p' DDE to lethal levels. All of the birds on a 100 ppm DDT diet and low temperature (-18 C) that died had between 21.6 and 26.3 ppm DDT in the brain, and the 1 bird surviving the test had only 6.1 ppm DDT in the brain. This supports the findings of other investigators that approximately 20-30 ppm DDT in the brain is a toxic level. Since all 4 of the birds on 100-ppm-DDT diets and low temperature (including the surviving bird) had relatively high levels of p,p' DDE in the brain it would seem that p,p' DDT was the more important residue causing death.

The study of the effects of DDT on the energetics of bobwhite suggested that low dietary levels of DDT could have detrimental effects on quail survival in their natural environment. The slight increase in metabolic rate concomitant with the downward shift (40 C to at least 35 C) in the upper end of the zone of thermal neutrality and a decreased ability to withstand hyperthermia, decrease the chances of survival when quail are exposed to high ambient temperatures. This becomes extremely important because the bobwhite is typically a bird of southern North America.

At first thought, it would seem that at low ambient temperatures the increased metabolism due to DDT would be beneficial, enabling the bird to maintain its body temperature. At 5 C the body temperature of experimentals was not significantly lower than at 10 C while in the controls it was lower. At low ambient temperature there is usually a scarcity of food. Assuming a respiratory quotient of 0.75 (fasted bird oxidizing fats) 1 cc of oxygen consumed/g/hr can be equated to 4.7 cal/g/hr. Based on this figure, nonacclimated bobwhite quail on a 10 - 150 ppm diet would have an increased metabolic requirement of 2.5 Kcal/day at an ambient temperature of 5 C. In the acclimated quail on 100-ppm-DDT diet the increased metabolic requirement due to DDT was 9 Kcal/day at ambient temperature of 5 C. The increased metabolic expenditure must be met either by increased food intake or increased metabolism of stored fat. The scarcity of food, especially in the northern range of the bobwhite during the winter months, would mean that the birds would probably have to metabolize stored fats which would further influence the bird's life by increasing circulating levels of DDT and its metabolites.

As pointed out in our study, if residue levels in the fats are high enough, the bird could die of DDT toxicity. Even without DDT poisoning, weight losses could be high enough in DDT-fed birds (due to the increased energy requirement) to decrease survival. Our results support the findings of Neave and Wright (21) on the effects of weather and DDT spraying on 2 ruffed grouse (Bonasa umbellus) populations. They stated that a synergistic effect between DDT spraying and temperature resulted in increased egg loss. The synergistic effect might develop because the DDT birds have a higher metabolic rate than the controls (even though controls had an elevated metabolic rate due to cold stress). Therefore, a laying bird (due to a much higher metabolism than a bird without DDT and cold stress) would metabolize more fat, which in turn would increase circulating levels of DDE which would affect egg development.

The quail in our tests were in individual cages and could not covey during extreme-cold stress experiments. When the temperature in the environmental room was lowered, the quail gave the covey call. It is possible then, that coveying is a form of behavioral temperature regulation and at -18 C the energy requirement might be less in quail that covey than in our experimental birds.

Table 1. Conversion of progesterone and testosterone to their metabolites by control and DDT fed quail, 1969. BW = body weight; TP = testosterone polar metabolite; T4 =testosterone; LTP = less testosterone polar metabolite; PP = progesterone polar metabolite; P4 = progesterone; MO = control male; FO = control female; FX = experimental female; MX = experimental male.

Date, sex and treatment*	% TP or PP	% T4 or P4	% LTP	% BW of the testes
718 FO	15.6	84.4		
729 FO	24.8	75.2		
730 FO	32.0	68.0		
715 MO	25.8	69.0	4.75	0.70
724 MO	7.77	89.0	3.14	1.0
716 MO	3.45	54.5	10.4	0.55
729 MO	7.31	83.1	9.5	1.15
730 MO	3.67	92.7	3.55	0.65
1217 MOA	29.5	61.8	8.4	0.023
1217 MOB	23.1	45.7	31.5	0.01
729 FX	47.0	53.0		
730 FX	50.5	49.5		
81 FXA	42.8	57.2		
81 FXB	67.3	32.7		
717 MX	23.2	70.06	5.9	0.69
724 MX	23.2	68.8	7.96	0.64
1218 MX	39.4	53.6	6.89	0.02
729 MX	7.58	84.7	7.7	0.55
730 MX	8.5	87.7	3.75	0.54
812 MXA	37.58	58.82	3.6	0.188
99 MXA	37.1	59.8	2.89	0.44
99 MXB	21.6	68.0	10.6	0.645
99 MXC	20.8	75.6	3.85	0.50
99 MXD	12.7	81.0	6.3	0.41
911 MXA	29.8	65.5	4.64	0.13
911 MXB	21.0	74.6	4.9	0.32
911 MXC	56.0	38.0	5.74	0.03
911 MXD	18.3	64.8	4.9	0.37

*Prefix numeral indicates month and date (718 = July 18); suffix letter (A, B, C, D) indicates different birds on same date.

Table 2. Results from low temperature stress. CF = control female; CM = control male; TF = 10 ppm DDT female; TM = 10 ppm DDT male; HF = 100 ppm DDT female; HM = 100 ppm DDT male; * = birds not tested for brain residues. Means are ± one standard deviation.

Bird and diet	Survival time (hours)	Body weight (grams)	Thyroid weight (grams)	I^{131} activity after 28 hrs. counts/min.	% I^{131} activity per gram	Brain weight (grams)	% Brain lipid	Brain levels of p,p' DDE	Brain levels of p,p' DDT (ppm)
1CF	168.58	129.8	0.0135	21,600	308	0.7536	3.65	1.88	---
2CM	169.33	149.9	0.0096	22,300	446	0.9807	5.81	0.06	---
3CM	169.83	203.9	0.0219	22,000	193	0.9485	6.01	0.06	
4CM*	170.50	176.0	0.0068	---	-	---	--	---	---
6CF	172.00	140.2	0.0146	28,200	370	0.9603	5.83	0.20	---
Means		159.9 ± 29.9	0.0132 ± 0.005		329 ± 92				
9TF	172.50	146.7	0.0160	46,100	550	0.8289	4.89	2.96	0.66
10TM*	173.08	199.6	0.0179	---	-	---	--	---	---
11TF*	174.00	173.9	0.0118	---	-	---	--	---	---
12TF	174.50	150.1	0.0076	34,250	870	1.0843	5.81	3.8	0.32
13TM	175.16	184.1	0.0088	21,400	467	0.9096	5.88	1.96	0.21
14TM	175.83	171.2	0.0129	31,200	466	1.1611	5.77	1.0	0.23
Means		170.8 ± 20	0.0125 ± 0.0036		588 ± 166				
18HF	50.50	177.3	0.0251	44,900	344	1.0759	5.34	88.0	23.0
21HM	73.75	166.2	0.0270	52,500	374	1.0913	5.82	102.0	21.6
22HF	168.00	166.1	0.0246	44,900	351	1.1000	5.55	42.7	6.10
23HM	28.00	171.8	0.0167	63,400	720	1.0758	5.34	63.0	26.3
Means		170.3 ± 5.3	0.0233 ± 0.0039		447 ± 157				

Table 3. Mean residues of DDT and its metabolites for 4 quail from each group. Feeding period was 211-242 days. Residues are given in parts per million (ppm) wet weight. Dash (-) indicates no detectable residues.

Group	Tissue	ppm DDT	ppm DDT	ppm DDE
control	Fat	-	-	1.5
N = 4	Brain	-	-	-
	Liver	-	-	0.3
10 ppm	Fat	11.12	-	41.37
N = 4	Brain	-	-	0.33
	Liver	-	-	2.96
50 ppm	Fat	-	-	283.0
N = 4	Brain	-	-	7.5
	Liver	-	-	17.5
100 ppm	Fat	238.1	-	929.5
N = 4	Brain	-	-	8.89
	Liver	-	-	17.9
150 ppm	Fat	791.25	-	1877.50
N = 4	Brain	3.07	-	10.87
	Liver	-	11.75	90.12

*All residues were p,p' isomers except for the brain residue in the controls which was o,p' DDE. Recovery levels averaged 73%.

Table 4. Results of dissections giving mean body and liver weights in grams of birds used in the oxygen consumption tests.

Group 1	Mean body weight	Mean liver weight	Mean % total body weight of liver
Controls	201.7 ± 3.6	3.5	1.74 ± 0.22
10 ppm	196.0 ± 18.6	3.5	1.77 ± 0.18
50 ppm	179.8 ± 14.5	4.3	2.38 ± 0.31
100 ppm	201.1 ± 11.3	5.1	2.54 ± 0.20
150 ppm	191.2 ± 12.4	4.2	2.22 ± 0.06

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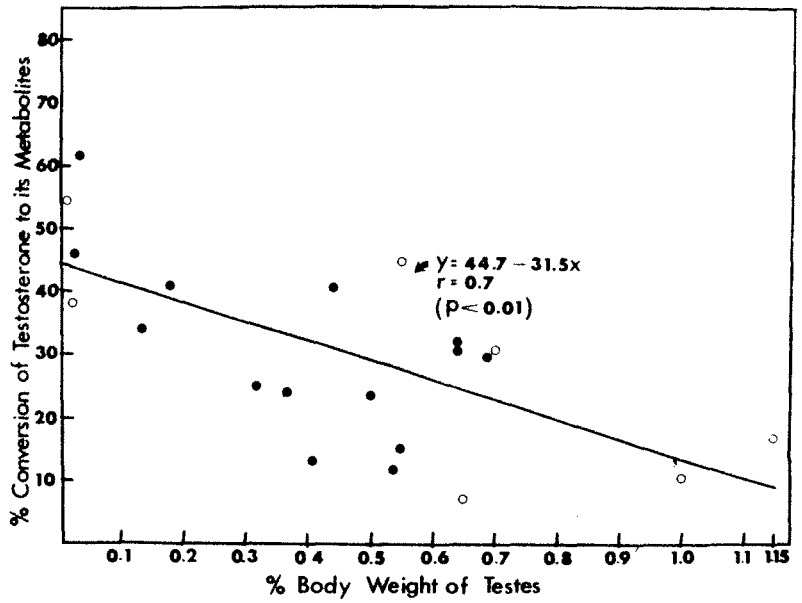

Figure 1. Relationship between testes weight and percent conversion of testosterone to its metabolites. The regression line is fitted by the method of least squares and has a correlation coefficient of r = 0.7 (P <0.01). Shaded circles represent experimental males, unshaded circles represent control males.

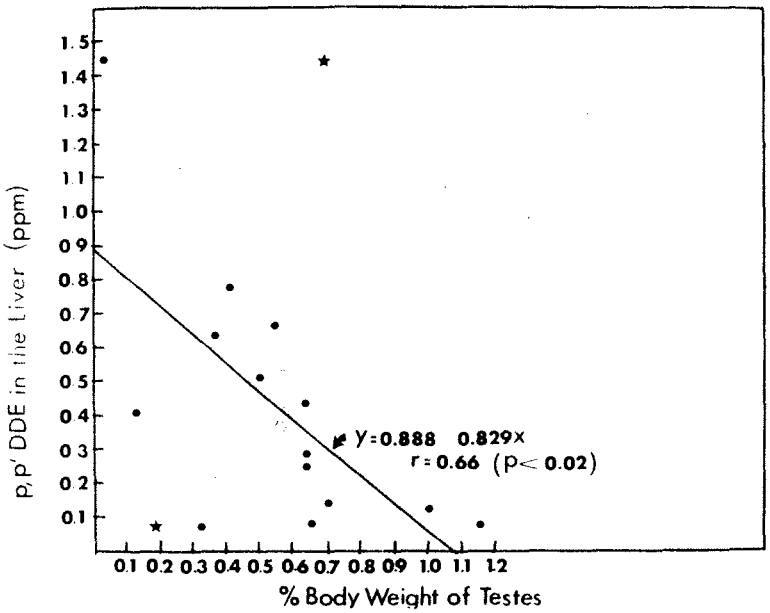

Figure 2. Relationship between percent body weight of testes and p,p' DDE in the liver, in experimental male bobwhite quail. The regression line is fitted by the method of least squares to the shaded circles and has a correlation coefficient of r = 0.66, P<0.02. The stars represent experimental birds not included in regression analysis.

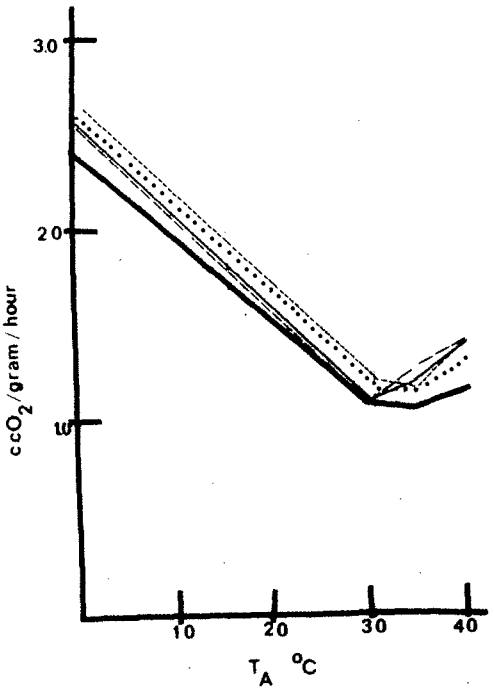

Figure 3. The slopes of the metabolic curves of the control group and 4 experimental groups below the lower critical temperature (-30° C). Means of the 5 groups are plotted at 35 and 40° C. n = 5. Using the least squares method to fit the standard metabolic curves to the lines, (Y = MX + b), the following results were obtained:

for control	2.4163 = (-0.0493)x + b	heavy solid line
10 ppm	2.5735 = (-0.0493)x + b	thin solid line
50 ppm	2.6885 = (-0.0494)x + b	dashed (short) line
100 ppm	2.6287 = (-0.0475)x + b	dotted line
150 ppm	2.5446 = (-0.0493)x + b	dashed (long) line

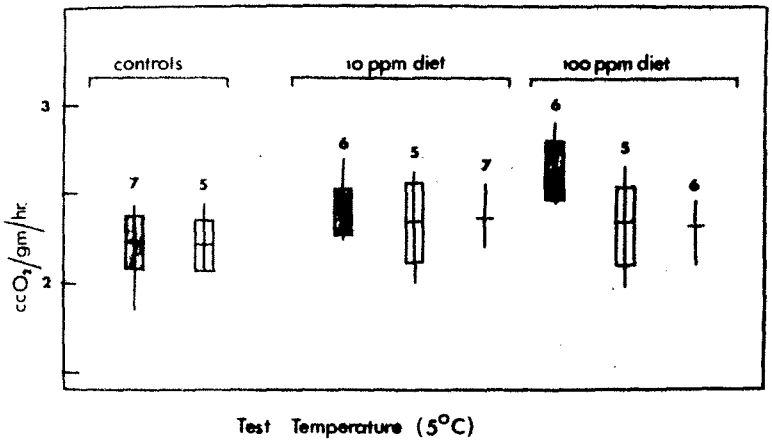

Figure 4. Comparison of the oxygen consumption at an ambient temperature of 5° C of quail acclimated for at least 10 weeks (shaded boxes), unacclimated quail (unshaded boxes), and quail acclimated for only 2 to 3 weeks (crosses) on three different diets (control, 10 and 100 ppm DDT). Vertical lines represent range, horizontal lines the mean and boxes the 95 percent confidence limits.

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DISEASES AND PARASITES OF THE BOBWHITE

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Abstract:

The authors' experiences with disease and parasite problems of wild and pen-raised bobwhites are given. Information extrapolated from an extensive literature review is presented in tabular form. Diseases and parasites of bobwhites, location in the bird of the lesion or parasite, and geographic areas of occurrence are note.

A variety of information concerning diseases and parasites of the bobwhite is available in the technical literature. The majority of mortality reports deal with pen-raised birds, but limited information is available concerning wild bobwhites. Basic publications related to diseases and parasites of bobwhites include: Biester and Schwarte's (1965) Diseases of Poultry; Davis, Anderson, Karstad, and Trainer's (1971) Infectious and Parasitic Diseases of Wild Birds; the section in Stoddard's (1931) book concerning diseases and parasites of the bobwhite; and Kellogg and Calpin's (1971) checklist of diseases and parasites reported from the bobwhite.

Today, 40 years after Stoddard's (1931) initial disclosure of diseases and parasites affecting quail, there is still little information